PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PCT-154		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/ES2004/070001	21.01.2004	28.01.2003

International Patent Classification (IPC) or both national classification and IPC

C12N15/09

Applicant

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1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24.08.2004	02.06.2005

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/ES2004/070001

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-56, 58-61	as originally filed
57	filed with telefax on 19.04.2005

Sequence listings part of the description, Pages

1-91	as originally filed

Claims, Numbers

1-14	received on 17.05.2005 with letter of 12.05.2005

Drawings, Sheets

1/3-3/3	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: English , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☒ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	7-12
	No:	Claims	1-6, 13 and 14
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations

see separate sheet

Present application relates to *in vitro* methods for detecting individuals who are predisposed to the disease named Familial Hypercholesterolemia (FH), and more particularly to a method for detecting the presence or absence of several mutations associated with FH. 54 gene mutations and polymorphisms that are all produced in the gene sequence of the low density lipoprotein receptor gene (LDL-r) set forth in SEQ ID NO:1 are disclosed. Microarrays comprising oligonucleotides that specifically detect any of said mutations, methods of diagnosis characterised in that at least one of said 54 mutations is detected in a biological sample as well as oligonucleotides specifically detecting any of the mutations comprised in the LDL-r gene sequence are claimed.

Re Item I

Basis of the report

I.1 The amendments filed with the letter dated 12.05.2005 appear to fulfill the requirements of Article 34(2)(b) PCT.

Re Item IV

Lack of unity of invention

The present application appears to lacks unity within the meaning of Rule 13.1 PCT. The following separate inventions can be considered:

a) Invention 1 (Claims 1 - 14; all partially):
Claims 1 - 14 relate to the mutation (-23)A> C in the LDL-r gene (SEQ ID NO:1). The subject-matter of said claims further encompasses oligonucleotides, respectively microarrays comprising oligonucleotides that specifically detect said mutation as well as methods for the detection of said mutation in a biological sample.

b) Inventions 2 - 54 (Claims 1 - 14; all partially):
As for Invention 1, but respectively relating to the mutations 1054del11, 108delC,.....[1587-5del15;1587del31] (i.e. Invention 2 corresponding to the mutation 1054del11; Invention 3 corresponding to the mutation 108delC.....; Invention 54 corresponding to the mutation [1587-5del15;1587del31]) and the subject matter relating to said mutation.

The 54 inventions are not so linked as to form a general inventive concept for the following reasons:

The problem to be solved by the present application can be seen as the detection of mutations in the LDL-r gene with the aim to use them in diagnosis of FH.
The solution to this problem is provided with the 54 mutations referred to in claim 1, respectively in claims 9 and 13.
However, this general concept is "*a priori*" not novel, since a number of LDL-r gene mutations in FH had been publicly known before the priority date of the present application (see ISR: e.g. D1: page 3, line 21 - page 5, line 13; D2: Table 1). Also, it had been publicly known before the priority date of the present application to detect lipid metabolic errors such as FH by noting these mutations.
Therefore and since no other technical feature can be distinguished which might link the subject matter of said claims, each of the above mentioned group of claims represents an independent invention.
Hence, the present application does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V.1 The following documents were taken into account:

D1: WO 02 06467 A1, respectively EP1304374

D2: FOUCHIER S. ET AL: 'The molecular basis of familial hypercholesterolemia in The Netherlands.' HUMAN GENETICS vol. 109, no. 6, December 2001, pages 602 - 615, XP002980736

The European Patent Application EP1304374 which has been published in accordance with Article 158(3) EPC corresponds to the PCT application WO0206467 published on the 24.01.2002. Hence, EP1304374 is validly considered as English translation of WO0206467.

V.2 Novelty (Article 33(1) and (2) PCT)

V2.1 Present application discloses/claims 259 oligonucleotide sequences (SEQ ID Nos: 1-259) that can be used to detect 54 mutations in the DNA sequence of the LDL-r gene (SEQ ID NO:1). With regard to the available prior art as cited in the ISR (S.P.T.O; no invitation to pay additional fees was issued) and under consideration that the expression "*..oligonucleotides able of **specifically** detecting...*" means that only

oligonucleotide sequences that are 100%identical to the mutation(s) as referred to in claim 13 are encompassed by the scope of said claim(s), the subject matter of claims 1 - 14 is considered as novel, since it is not anticipated by the available prior art. Hence, it complies with the requirements of Article 33(1) and (2) PCT.

V.3 Inventive Step (Article 33(1) and (3) PCT)

V3.1 The technical contribution of claims 1 - 6, 13 and 14 can be summed up as the provision 54 mutations found in the LDL-r gene as referred to in claim 1, respectively the provision of 259 oligonucleotide sequences that can be used for the detection of said mutations.

With regard to prior art (D1 and D2 already disciose mutations in the LDL-r gene and methods for the detection of the same; D1: page 3, line 21 - page 5, line 13; D2: Table 1), the technical problem solved by the present application is the provision of alternative mutations to be found in the LDL-r gene and the provision of oligonucleotides that can be used for the detection of said mutations.

The 54 mutations as referred to in claim 1 are found out merely by comparing the base sequence of the normal LDL-r gene with the base sequences of LDL-r genes of patients clinically diagnosed as suffering form FH. Hence, taking into consideration the prior art in combination with general knowledge, the provision of further gene sequences comprising mutations in the LDL-r gene, respectively the provision of oligonucleotide sequences that specifically detect said mutations, would be obvious and straight forward for the person skilled in the art.

Moreover, the IPEA raises the Applicant's attention to the fact that with regard to the statement of D2, namely that FH patients from different populations are characterised by different mutations in the LDL-r gene (D2: page 611, column 2, paragraph 2 - page 612, column 1, paragraph 4), it could be further expected that screening of Spanish FH cases would reveal different mutations compared to the ones obtained by screening Dutch (D2) and/or Japanish FH samples (D1).

Thus, it is not possible to acknowledge inventive step for the subject-matter referred to in 13 and 14.

The subject-matter of claims 1 - 6 that refers to microarrays comprising oligonucleotides for the detection of the 54 mutations in the LDL-r gene (SEQ ID NO:1) is also not considered to be inventive since with regard to general knowledge the coupling of an oligonucleotide to a solid support, respectively the provision of a microarray comprising said oligonucleotide sequence cannot be regarded as

sufficient to establish inventive activity.
Hence, claims 1 - 6, 13 and 14 are therefore considered to lack inventive step under Article 33(3) PCT.

V3.2 Claims 7 and 8 relate to the use of oligonucleotides in extracorporeal methods of in vitro detection of the LDL-r gene mutations for diagnosis of FH and the subject-matter fo claims 9 - 12 relate to extracorporeal methods of diagnosis of FH characterized in that in a biological sample at least one mutation in the LDL-r gene as referred to is detected.
D1 that can be considered as closest prior art for evaluating the inventiveness of the subject-matter referred to in claims 7 - 12 discloses methods for the detection of mutations in the LDL-r gene (e.g.: page 3, line 21 - page 5, line 13).
Present application differs from D1 in that the method is characterised by mutations that were found in the Spanish population whereas D1 relates to methods that are based on mutations to be found in the Japanese population. Hence, the technical problem to be solved can be seen as the provision of a method to diagnose FH in the Spanish population.
Since the available prior art neither discloses nor suggests a method for the diagnosis of FH caused by different mutations in the Spanish population and since apparently the claimed method enables a very specific system for the diagnosis of FH in Spain, the subject-matter referred to in claims 7 - 12 appears to be inventive (Article 33(3) PCT).

V.4 Industrial Applicability (Article 33(1) and (4) PCT)

V4.1 The subject matter of claims 1 - 14 is considered industrially applicable. Hence, it meets the requirements of Article 33(1) and (4) PCT.

Certain Observations on the International Application

*The following remarks on **Clarity and Sufficiency of Disclosure** (Article 6 and 5 PCT) are made:*
*1) Claims 1, 13 and 14 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined (...oligonucleotides **able of specifically detecting....**). The claims attempt to define the subject-matter in terms of the result to be achieved, which merely amounts to a statement of the underlying problem, without*

providing the technical features necessary for achieving this result.

AMENDED CLAIMS

1.- Microarray characterized by comprising oligonucleotides able of specifically detecting in the DNA sequence of LDL-r gene (SEQ ID NO: 1) any of the mutations selected from: (-23)A>C, 1054 del11, 108delC, 1197de19, 1207de1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+1insT, 338del16, 509insC, 675del15, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818de18, 1423delGC/insA, 1204insT, 451de13, G516X, 2389+4A>G, 1815del11, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31].

2.- Microarray according to claim 1 characterized by comprising at least an oligonucleotide selected from: SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:29, or at least one from SEQ ID NO:37 to SEQ ID NO:147 or from SEQ ID NO:154 to SEQ ID NO:259.

3.- Microarray according to any of the claims 1 or 2 characterized by further comprising oligonucleotides able of specifically detecting in the DNA sequence of LDL-r gene (SEQ ID NO: 1) any of the mutations selected from: 2393del9, (-42)C>G, (-49)C>T, 1045delC, 1061-8 T>C, A378T, C358R, 1358+1G>A, 1706-10G>A, 1845+1G>C, 2085del19, 2111delG, 2140+5G>A, 2207insT, 2390-1G>C, 313+1G>C, 313+1G>A, 518delG, 7delC, 872delC, 884delT, 920ins4, A519T, C113W, C255X, C281Y, C297F, C347Y, C371X, C646Y, C677Y, C68W, C74G, C95R, D151N, D200G, D200Y, D280G, E10X, E246A, E256K, F634L, G322S, G352D, G571E, N543H, N804K, Q12X, Q133X, Q357P, Q427X, Q71E, R395Q, R574W, R612C, S156L, S205P, T413K, T705I, V502M, W(-18)X, W541X, D679E, 1359-1G>A, C127R, 681ins21, C122X, V408M, G528D, D412H, N619N, E80K, L534P, L621S, C356Y, R329X, G248D, C201Y,

313+5G>A, C358Y, C331R, D157N, V776M, P664L, W462X, Q328X, L584P, R395W, G314V, W469X, P678L, R612H, R236W.

4.- Microarray according to any of the claims 1 to 3 characterized by further comprising oligonucleotides able of specifically detecting in the DNA sequence of LDL-r gene (SEQ ID NO: 1) any of the polymorphims selected from: 81T>C BstUI Exon 2, 1060+10G>C SmaI Exon 7, 1171G>A StuI Exon 8, 1413G>A DdeI Exon 10, 1617C>T BstNI Exon 11, 1725C>T SSCP Exon 12, 1771C>T HincII Exon 12, 1959 T>C AvaII Exon 13, 2232G>A MspI Exon 15.

5.- Microarray according to any of the claims 1 to 4 characterized by comprising at least an oligonucleotide selected from: SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:153.

6.- Microarray according to any of the claims 1 to 5 characterized by having the oligonucleotides coupled to a support.

7.- Use in extracorporeal methods of detection of mutations in LDL-r gene (SEQ ID NO: 1) for in vitro diagnosis of familial hypercholesterolemia of any of the oligonucleotides selected from: SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:29, or at least one from SEQ ID NO:37 to SEQ ID NO:147 or from SEQ ID NO:154 to SEQ ID NO:259.

8.- Use in extracorporeal methods of detection of mutations in LDL-r gene (SEQ ID NO: 1) for in vitro diagnosis of familial hypercholesterolemia, according to claim 7 of any of the oligonucleotides selected from: SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:29, or at least one from SEQ ID NO:37 to SEQ ID NO:147 or from SEQ ID NO:154 to SEQ ID NO:259, in combination with any of the oligonucleotides selected from: SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO: 9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:153.

9.- Extracorporeal method of in vitro diagnosis of familial hypercholesterolemia characterized in that in a biological sample of an individual is detected in LDL-r gene (SEQ ID NO: 1), at least one mutation selected from: (-23)A>C, 1054 del11, 108delC, 1197de19, 1207de1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+1insT, 338del16, 509insC, 675del15, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818de18, 1423delGC/insA, 1204insT, 451de13, G516X, 2389+4A>G, 1815del11, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31].

10.- Extracorporeal method of in vitro diagnosis of familial hypercholesterolemia, according to claim 9, characterized in that in a biological sample of an individual, in combination with at least one of the mutations in LDL-r gene (SEQ ID NO: 1) selected from: (-23)A>C, 1054 del11, 108delC,

1197del9, 1207del1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+linsT, 338dell6, 509insC, 675dell5, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818del8, 1423delGC/insA, 1204insT, 451del13, G516X, 2389+4A>G, 1815dell1, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5del5;1587del31] is further detected, in the same LDL-r gene (SEQ ID NO: 1), at least one mutation selected from: 2393del9, (-42)C>G, (-49)C>T, 1045delC, 1061-8 T>C, A378T, C358R, 1358+1G>A, 1706-10G>A, 1845+1G>C, 2085del19, 2lldelG, 2140+5G>A, 2207insT, 2390-1G>C, 313+1G>C, 313+1G>A, 518delG, 7delC, 872delC, 884delT, 920ins4, A519T, C113W, C255X, C281Y, C297F, C347Y, C371X, C646Y, C677Y, C68W, C74G, C95R, D151N, D200G, D200Y, D280G, E10X, E246A, E256K, F634L, G322S, G352D, G571E, N543H, N804K, Q12X, Q133X, Q357P, Q427X, Q71E, R395Q, R574W, R612C, S156L, S205P, T413K, T7051, V502M, W(-18)X, W541X, D679E, 1359-1G>A, C127R, 681ins21, C122X, V408M, G528D, D412H, N619N, E80K, L534P, L621S, C356Y, R329X, G248D, C201Y, 313+5G>A, C358Y, C331R, D157N, V776M, P664L, W462X, Q328X, L584P, R395W, G314V, W469X, P678L, R612H, R236W.

11.- Extracorporeal method of in vitro diagnosis of familial hypercholesterolemia, according to any of the claims 9 or 10, characterized in that in a biological sample of an individual, in combination with at least one of the mutations in LDL-r gene (SEQ ID NO: 1) selected from: (-23)A>C, 1054 del11, 108delC, 1197del9, 1207del1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+linsT, 338dell6, 509insC, 675dell5, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818del8, 1423delGC/insA, 1204insT, 451del13, G516X, 2389+4A>G, 1815dell1, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E,

L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31], 2393del9, (-42)C>G, (-49)C>T, 1045delC, 1061-8 T>C, A378T, C358R, 1358+1G>A, 1706-10G>A, 1845+1G>C, 2085del19, 2lldelG, 2140+5G>A, 2207insT, 2390-1G>C, 313+1G>C, 313+1G>A, 518delG, 7delC, 872delC, 884delT, 920ins4, A519T, C113W, C255X, C281Y, C297F, C347Y, C371X, C646Y, C677Y, C68W, C74G, C95R, D151N, D200G, D200Y, D280G, E10X, E246A, E256K, F634L, G322S, G352D, G571E, N543H, N804K, Q12X, Q133X, Q357P, Q427X, Q71E, R395Q, R574W, R612C, S156L, S205P, T413K, T7051, V502M, W(-18)X, W541X, D679E, 1359-1G>A, C127R, 681ins21, C122X, V408M, G528D, D412H, N619N, E80K, L534P, L621S, C356Y, R329X, G248D, C201Y, 313+5G>A, C358Y, C331R, D157N, V776M, P664L, W462X, Q328X, L584P, R395W, G314V, W469X, P678L, R612H, R236W, is further detected at least one LDL-r gene (SEQ ID NO: 1) polymorphism selected from: 81T>C BstUI Exon 2, 1060+10G>C SmaI Exon 7, 1171G>A StuI Exon 8, 1413G>A DdeI Exon 10, 1617C>T BstNI Exon 11, 1725C>T SSCP Exon 12, 1771C>T HincII Exon 12; 1959 T>C AvaII Exon 13, 2232G>A MspI Exon 15.

12.- Extracorporeal method of in vitro diagnosis of familial hypercholesterolemia according to any of the claims 9 to 11, comprising amplifying DNA fragments that contain any mutation in LDL-r gene (SEQ ID NO: 1) selected from: (-23)A>C, 1054 del11, 108delC, 1197de19, 1207de1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+linsT, 338dell6, 509insC, 675dell5, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818de18, 1423delGC/insA, 1204insT, 451de13, G516X, 2389+4A>G, 1815del11, 1186+5G>A, T740M, I771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31], alone or in combination with any mutation in LDL-r gene (SEQ ID NO: 1) selected from: 2393del9, (-42)C>G, (-49)C>T, 1045delC, 1061-8 T>C, A378T, C358R, 1358+1G>A, 1706-10G>A, 1845+1G>C, 2085del19,

2lldelG, 2140+5G>A, 2207insT, 2390-1G>C, 313+1G>C, 313+1G>A, 518delG, 7delC, 872delC, 884delT, 920ins4, A519T, C113W, C255X, C281Y, C297F, C347Y, C371X, C646Y, C677Y, C68W, C74G, C95R, D151N, D200G, D200Y, D280G, E10X, E246A, E256K, F634L, G322S, G352D, G571E, N543H, N804K, Q12X, Q133X, Q357P, Q427X, Q71E, R395Q, R574W, R612C, S156L, S205P, T413K, T7051, V502M, W(-18)X, W541X, D679E, 1359-1G>A, C127R, 681ins21, C122X, V408M, G528D, D412H, N619N, E80K, L534P, L621S, C356Y, R329X, G248D, C201Y, 313+5G>A, C358Y, C331R, D157N, V776M, P664L, W462X, Q328X, L584P, R395W, G314V, W469X, P678L, R612H, R236W and/or any polymorphism in LDL-r gene (SEQ ID NO: 1) selected from: 81T>C BstUI Exon 2, 1060+10G>C SmaI Exon 7, 1171G>A StuI Exon 8, 1413G>A DdeI Exon 10, 1617C>T BstNI Exon 11, 1725C>T SSCP Exon 12, 1771C>T HincII Exon 12, 1959 T>C AvaII Exon 13, 2232G>A MspI Exon 15, by the technique of the chain reaction of the polymerase (PCR), utilizing therefore any of the oligonucleotides selected among SEQ ID NO:2 to SEQ ID NO:259 or combinations of the same, subjecting the PCR products to an analysis by the simple chain conformation polymorphisms technique (SSCP), sequencing those fragments having an anomalous pattern by SSCP to detect the mutations, that would be identified subsequently by restriction analysis or by means of the microarray of claims 1 to 6.

13.- Oligonucleotides able of specifically detecting in LDL-r gene (SEQ ID NO: 1) any of the mutations selected from: (-23)A>C, 1054 del11, 108delC, 1197de19, 1207de1T, 1432delG, 191-2delAinsCT, 2184delG, 231delC, 2399del5ins4, 313+linsT, 338dell6, 509insC, 675dell5, 684dup12, 941-39>T, C195R, C255G, C319Y, D157G, D630N, E291X, H635N, N59K, T41M, W515X, Y379X, Y421X, T433N, 818de18, 1423delGC/insA, 1204insT, 451de13, G516X, 2389+4A>G, 1815del11, 1186+5G>A, T740M, 1771T, R279G, T446I, H562Q, C74Y, D686Y, G(-2)R, E579D, S205C, D200V, V766E, L(-6)P, 2544insC, C42Y, 2389+3A>C, [1587-5de15;1587del31].

14.- Oligonucleotides according to claim 13 selected from: SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:29, or at least one from SEQ ID NO:37 to SEQ ID NO:147 or from SEQ ID NO:154 to SEQ ID NO:259.

fragments were electrophoresed in 8% polyacrilamide gel and were visualized by staining with ethidium bromide. Alternatively, this mutation could be analyzed with the microarray ("biochip") by spotting onto the slide the oligonucleotides SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198 y SEQ ID NO: 199.

I771T mutation was detected in a 60 years old woman from a family with autosomal dominant hypercholesterolemia and history of premature cardiovascular disease She has been diagnosed as having familial hypercholesterolemia with a MedPed diagnostic score of 21 points. Her plasma lipid levels were: TC (422 mg/dL) and LDLc (368 mg/dL), and normal TG and HDLc levels

2389+3 A>C mutation analysis

This mutation 2389+3 A>C was identified by DNA sequencing of the 273 pb fragment from exon 16 during screening for mutations in the LDL-r gene in subjects clinically diagnosed as having FH. Purified PCR product from DNA sample were directly sequenced in both directions using the amplification primers Ex 16F (SEQ ID NO: 152) y EX16R (SEQ ID NO: 153). and the kit CEQ 2000 Dye Terminator Cycle Sequencing with Quick Start (Beckman Coulter, Palo Alto, CA, USA) according to the protocol described by the manufacturer. Sequences were detected using the CEQ 8000 Genetic Analysis System (Beckman Coulter, Inc. Fullerton), and analyzed with CEQ 8000 software. The A>C change was confirmed by sequencing a second PCR product. Alternatively, this mutation could be analyzed with the microarray ("biochip") by spotting onto the slide the oligonucleotides: SEQ ID NO: 252, SEQ ID NO: 253, SEQ ID NO: 254 y SEQ ID NO: 255.

2389+3 A>C mutation was detected in a 36 years old man with multiple tendon xantomas and family history of hypercholesterolemia. He was diagnosed clinically as having FH with a MedPed score of 18 points. Analysis of her fasting serum lipid levels without use of lipid lowering therapy were: TC (450mg/dL) with normal TG and HDLc levels. Lipid lowering treatment with atorvastatin (20mg/dL) reduced his LDL-c to 259 mg/dL